Influence of Sodium Chloride on Thermal Inactivation and Recovery of Nonproteolytic *Clostridium botulinum* Type B Strain KAP B5 Spores†

ABSTRACT

Demand for minimally processed refrigerated foods with reduced salt levels has stimulated renewed interest in the potential for survival and growth of psychrotrophic, nonproteolytic *Clostridium botulinum* type B spores. As part of a project to better define food-processing requirements, the heat resistance (75 to 90°C) of nonproteolytic *C. botulinum* type B spores was assessed in turkey containing 1 to 3% (wt/vol) salt (sodium chloride). Heated spores were recovered both on reinforced clostridial medium (RCM) with lysozyme and on RCM having the same salt levels as the heating menstruum. When the recovery medium contained no salt, D-values in turkey slurry containing 1% salt were 42.1, 17.1, 7.8, and 1.1 min at 75, 80, 85, and 90°C, respectively. Increasing levels (2 and 3%, wt/vol) of salt in the turkey slurry reduced the heat resistance as evidenced by reduced spore D-values. Also, apparent or measured heat resistance was decreased with increasing salt concentration in the heating menstruum and the recovery medium. The z-values in turkey slurry for all treatments were similar, ranging from 8.47 to 10.08°C. These data will assist food processors to design thermal processes that ensure safety against nonproteolytic *C. botulinum* type B spores in cook/chill foods while minimizing quality losses.

Key words: *Clostridium botulinum*, spores, thermal inactivation, sodium chloride

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Clostridium botulinum spores occur naturally in soil and are potential contaminants in minimally processed food products that rely solely on refrigeration for extended storage *(16, 26)*. While proteolytic type A and B strains of *C. botulinum* produce fairly heat-resistant spores *(15)* and have a minimal growth temperature of 10°C *(25)*, nonproteolytic type B strains form considerably less heat-resistant spores *(15, 22)* and can grow at temperatures as low as 3.3°C *(23, 24)*. Thus, spores of nonproteolytic strains that survive the thermal process would pose a botulism hazard even under proper refrigeration temperatures, if a secondary barrier was not present.

Nonproteolytic *C. botulinum* type B has been implicated in food-borne botulism outbreaks in humans after consumption of kapchunka *(2)*, fermented fish products in Canada and Alaska *(6, 7)*, and from a number of products such as canned meats and vegetables, ham, and fermented sausages in Europe *(14, 27)*. The spores of this organism have been found in marine sediments and in fresh-water environments, including fresh-water fish *(9, 18)*. The heat treatment required to achieve a specified lethality for nonproteolytic *C. botulinum* type B spores in minimally processed foods must be well defined to ensure that the heating step is lethal, while avoiding heating that negatively impacts product quality.

Salt, after sugar, is the second most used food additive in the food-processing industry *(1)*. While the effect of increased salt levels in recovery media on the reduced recovery of heat-injured spores has been documented for *Bacillus stearothermophilus, C. sporogenes*, and proteolytic *C. botulinum (3, 5, 20, 21)*, there appears to be no work available with respect to nonproteolytic *C. botulinum* type B spores. Accordingly, the present study was undertaken to determine the effect of 1 to 3% salt in turkey on nonproteolytic *C. botulinum* type B spores "apparent" heat resistance.

MATERIALS AND METHODS

Preparation of spore suspension and turkey slurry

Spores of nonproteolytic *C. botulinum* type B strain KAP B5 were produced in trypticase peptone glucose yeast extract by the procedure described previously *(12)*. A known weight of ground turkey was aseptically transferred to a sterile Waring Blender, mixed with an equal volume of sterile water and blended for 2 min to form a smooth paste. Salt was added to the turkey slurry, which was again blended to ensure even distribution of salt and to obtain final levels of 1, 2, or 3% (wt/vol).

Thermal treatment of spore suspensions

Turkey slurry containing 1, 2, or 3% (wt/vol) salt was inoculated with heat-shocked (60°C, 10 min) spores to obtain an initial count of about 7 $\log_{10}$ spores per ml. Thermal inactivation was carried out at 75, 80, 85, or 90°C in sterile 17 by 60 mm screw-cap vials as described previously *(12)*. The duration of time of the heat treatments was based on the temperature being studied and ranged from 15 to 45 min. The surviving population of spores was determined by spiral plating (Model D, Spiral Systems, Cincinnati, OH) on agar plates containing RCM supplemented with lysozyme and on RCM containing lysozyme and the same percentage of salt as in the heating menstruum. The plates were incubated anaerobically at 28°C for 6 days for recovery of heat-injured spores. The D-values (time for a 10-fold reduction in viable spores) were determined by a survival model *(13, 28)* that was fitted to the experimental data using a Gauss-Newton curve-fitting program (ABACUS Software Program, ERRC, USDA, Philadelphia, PA). The z-values were estimated by computing the linear regression *(17)* of $\log_{10}$ D-values versus heating temperatures using Lotus 1-2-3 software.

RESULTS AND DISCUSSION

The "apparent" heat resistance (expressed as D-values in min) of nonproteolytic *C. botulinum* type B strain KAP B5 spores in turkey slurry containing 1% salt heated at 75 to 90°C ranged from 42.1 to 1.1 min when RCM supplemented with lysozyme was used as the recovery medium for heat-damaged spores (Table 1). In contrast, the estimated D-values obtained for spores of the same organism recovered on the same medium after heating in turkey slurry in the absence of salt ranged from 32.5 min at 75°C to 0.8 min at 90°C *(12)*. The differences in the D-values derived from spore recoveries on the same medium may be attributed to the presence or absence of 1% salt in the heating menstruum.

The protective effect of salt leading to increased D-values has been reported previously *(19)*. Inclusion of salt in the heating menstruum results in reduced water activity leading to spore dehydration, which accounts for the increased heat resistance. Gould and Dring *(10)* suggested that developing and maintaining extreme thermal resistance of bacterial endospores depends on the ability to maintain relatively dry protoplasts through an osmotic function of the surrounding expanded cortex. Increasing levels (2 and 3%, wt/vol) of salt in the turkey slurry reduced heat resistance and the effect was quantified as reduced spore D-values (Table 1). While these results are similar to those of Cook and Gilbert *(5)* and Briggs and Yazdany *(3)*, who reported that the heat resistance of *B. stearothermophilus* spores was progressively reduced with increasing concentrations (2, 4 and 8% wt/vol) of salt in the heating medium, the reason for decreased heat resistance at > 1% salt levels remains unexplained and needs to be investigated. With RCM containing lysozyme as the recovery medium, the z-values calculated from D-values obtained in turkey slurry with 1 to 3% salt, in the present study, ranged from 9.21 to 9.86 (Figure 1). These values are very similar to that calculated from D-values obtained in the absence of salt in turkey (z-value of 9.43°C; *[12]*).

TABLE 1. *Heat resistance (75 to 90°C), expressed as D-values in min, for nonproteolytic* Clostridium botulinum *type B strain KAP B5 in turkey slurry. The recovery medium was reinforced clostridial medium with lysozyme (RCM + L).*

Heating Menstruum	Temperatures (°C)			
	75	80	85	90
Turkey + 1% salt	42.1[a]	17.1	7.8	1.1
Turkey + 2% salt	25.7	15.1	5.5	0.6
Turkey + 3% salt	17.7	13.1	3.2	0.5

The D-values were 27.4, 13.2, 5.0, and 0.8 min at 75, 80, 85, and 90°C, respectively, when both the turkey slurry and the recovery medium contained 1% salt (Table 2). Increasing levels (2 to 3%, wt/vol) of salt in turkey slurry resulted in parallel decreases in the D-values obtained from the recovery of spores on media containing the same levels of salt as the heating menstruum (Table 2).

The decrease in D-values obtained from the recovery of heat-damaged spores on the media with added salt was due to the inability of heat-injured spores to recover in the presence of salt. The heat-injured spores were sensitive to salt in the recovery medium. Hutton et al. *(11)* studied the action of both pH and sodium chloride on heat resistance of *C. botulinum* 213B spores and obtained similar results. In their study, the presence of salt at 2% levels in the modified PA3679 agar decreased the spore D-values by 20 to 40% irrespective of the pH of the heating menstruum. The z-values calculated over the temperature range 75 to 90°C for spores suspended in turkey slurry containing 1 to 3% salt ranged from 8.47 to 10.08°C (Figure 2).

For growth of nonproteolytic *C. botulinum* type B to occur in the new generation of precooked (pasteurized) refrigerated foods, four criteria must be met: activation, germination, outgrowth of spores, and vegetative growth. The spore may be sensitive to the effects of salt during

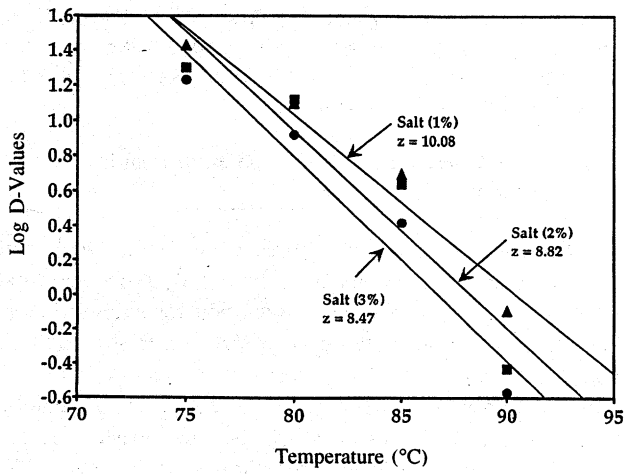

Figure 1. *Thermal-death-time curves (z-values) for nonproteolytic* C. botulinum *type B strains KAP B5 over the temperature range 75 to 90°C. The D-values, obtained in turkey slurry containing 1 to 3% salt, used to determine the z-values were the means of two replicates and were obtained based on survivors on reinforced clostridial medium (RCM) with lysozyme (L).*

TABLE 2. *Heat resistance (expressed as D-values in min) for nonproteolytic* Clostridium botulinum *type B strain KAP B5 between 75 and 90°C.*

Heating Menstruum	Recovery Medium	Temperatures (°C)			
		75	80	85	90
Turkey + 1% salt	RCM[a] + L + 1% salt	27.4[b]	13.2	5.0	0.8
Turkey + 2% salt	RCM + L + 2% salt	19.9	12.6	4.3	0.4
Turkey + 3% salt	RCM + L + 3% salt	16.9	8.2	2.6	0.3

[a] Reinforced clostridial medium.
[b] D-values are the means of two replicates.

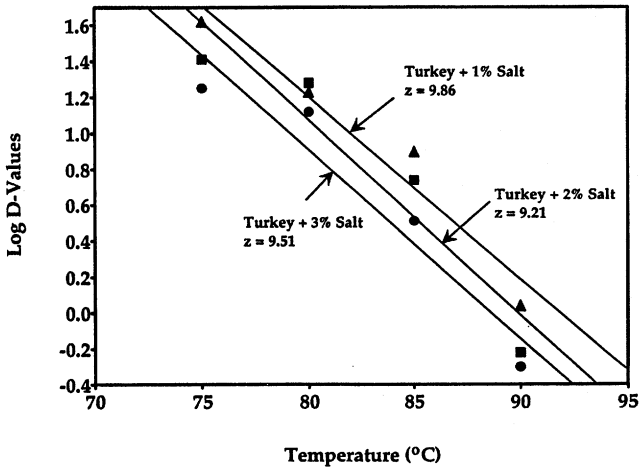

Figure 2. *Thermal-death-time curves (z-values) for nonproteolytic* C. botulinum *type B strains KAP B5 over the temperature range 75 to 90°C. The D-values, obtained in turkey slurry that included 1 to 3% salt, used to determine the z-values were the means of two replicates and were obtained based on survivors on reinforced clostridial medium (RCM) with lysozyme (L) and the same salt levels as in the turkey.*

either DNA repair, germination, outgrowth, or cell division *(4, 8)*. Thus, salt may be interfering with a stage in the transition of injured spores to metabolically active vegetative cells. In the present study, the ability of injured spores to recover was improved when salt was not included in the recovery medium and the spores were heated in turkey containing 1, 2 or 3% (wt/vol) salt.

The present study suggests that salt in concentrations > 2% (wt/vol) may be added to turkey to decrease the heat resistance of nonproteolytic *C. botulinum* type B spores. Also, our study simulated the conditions in minimally processed refrigerated foods and suggests that the inclusion of 1 to 3% (wt/vol) salt in turkey will decrease the duration of the heat treatment required as observed by decreased spore recovery. While Juneja et al. *(12)* reported that contaminated turkey should be heated to an internal temperature of 80°C for at least 91.3 min to give a 6-D process for type B spores, we concluded that with the inclusion of 3% salt, 78.6 min at 80°C would be sufficient to achieve a 6-D process. Thermal-death-time values from this study will assist food processors to include low levels of salt while formulating foods and to design a reduced thermal process that ensures safety against nonproteolytic *C. botulinum* type B in minimally processed foods while maintaining their desirable organoleptic attributes.

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